SUBGROUP: Membrane Structure & Assembly

16-Subg
Fluid-Fluid Phase Separation in Cholesterol-Containing Membranes: Rafts and Non-Rafts
Derek Marsh.
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Fluid-fluid phase separation is the functionally important mode of in-plane domain formation in lipid-bilayer membranes. The most common, if not sole, well-established case of fluid phase separation is that between liquid-ordered (L_o) and liquid-disordered (L_α) phases in cholesterol-containing membranes. The liquid-ordered phase is characterised by short-range orientational order, but long-range translational disorder. It is well documented for gel-fluid (L_α-L_o) phase coexistence in binary lipid mixtures with cholesterol but, surprisingly, direct evidence for the functionally significant fluid-fluid (L_α-L_o) phase coexistence is rather sparse. Interestingly, evidence for L_α-L_o coexistence is more abundant in ternary mixtures of a high-chain-melting lipid and a low-chain-melting lipid with cholesterol than in binary mixtures. Here, I shall review the current status of the field: it seems that no aspect is without some controversy. Published phase diagrams for the canonical "raft" lipid mixtures, *N*-palmitoyl sphingomyelin/palmitoyl-oleoyl phosphatidylcholine/cholesterol will be compared, and also new spin-label EPR results on this system will be presented.
Marsh, D., 2009. Cholesterol-induced fluid membrane domains: a compendium of lipid-raft ternary phase diagrams, Biochim. Biophys. Acta 1788, 2114-2123.
Marsh, D., 2010. Liquid-ordered phases induced by cholesterol: a compendium of binary phase diagrams, Biochim. Biophys. Acta 1798, 688-699.

17-Subg
Phospholipid Complexation of General Anesthetics: A Wake-Up Call
Steven L. Regen, Serhan Turkyilmaz.
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General anesthesia represents one of the most important advances in the history of medicine. Despite numerous attempts to determine how general anesthetics function at the molecular level, it remains to be established whether signaling proteins or the surrounding lipids serve as their primary targets. In this talk, I will discuss recent studies that we have carried out in which the effects of chloroform on phospholipid-sterol interactions in the liquid-ordered (lo) and the liquid-disordered (ld) phases have been quantified via the nearest-neighbor recognition (NNR) method. Using host membranes made from 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol, evidence has been obtained for 1/1 complexes being formed from chloroform and DPPC in both phases. At clinically-relevant concentrations (ca. 2 mM), chloroform was found to weaken cholesterol_DPPC interactions in the lo phase by 24.5 $\pm$ 5.5 cal/mol of lipid, while no effect could not be detected in the ld phase. Increasing the concentration of chloroform resulted in a convergence of nearest-neighbor interaction energies and the apparent formation of a common ''liquid-anesthetic'' (la) phase. The implications of these findings with regard to modern lipid theory of anesthesia will be discussed.

18-Subg
The Physics of Nerves and Lipid Membrane Channels
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At physiological temperature, many biomembranes are found in a physical state slightly above the melting transition of the membrane lipids. We show that this condition gives rise to the possibility of the propagation of electromechanical pulses (solitons) in membrane cylinders that share many properties with the action potential in nerves. Among those properties are the reversible heat change measured in nerves under the influence of the action potential, a mechanical shortening and thickening of the nerve, but also the excitability by voltage, local cooling, and by mechanical stimulus. Depending on boundary conditions one can obtain voltage pulse trains with minimum pulse distance (refractory periods) and undershoot (hyperpolarization).
The underlying physics is that of the fluctuation-dissipation theorem, that contains strict thermodynamic couplings between heat capacity, compressibility and lifetimes of membrane processes. It contains a role for all thermodynamic variables (not only voltage). We show that our electromechanical picture also contains a mechanism for anesthesia that lies in the melting point depression caused by these drugs, in agreement with the well-known Meyer-Overton correlation for anesthetics. The presence of anesthetics results in the reduction of the membrane excitability. Further, a direct consequence of the membrane fluctuations is the observation of spontaneous formation of ion-channel-like conduction events in the pure lipid membrane indistinguishable from those attributed to ion channel proteins. Since the theory is of macroscopic thermodynamic nature it does not make statements on processes on the molecular scale. However, it is consistent with known pharmacology provided that the macroscopic conservation of heat is maintained.
Summarizing, we provide thermodynamic picture for many excitatory processes in biomembranes that let pulse propagation, ion conductance, and action of drugs all be part of one self-consistent physical description.

19-Subg
Amphipols as a Strange but Efficient Medium in Which to Fold Membrane Proteins: Applications and Implications
Jean-Luc POPOT.
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Amphipols (APols) are short amphipathic polymers designed to substitute to detergents for handling membrane proteins (MPs) in aqueous solutions. Upon trapping a MP with APols, a non-covalent but stable complex forms, which is hydrosoluble and in which the MP is, in general, more stable than in detergent solution. In MP/APol complexes, the polymer covers the hydrophobic transmembrane surface of the protein, leaving extramembrane surfaces free to interact with water-soluble ligands. Functional perturbations appear to be rare.
The applications of APols that have been validated to date include stabilizing fragile MPs and MP complexes, solution NMR studies, electron microscopy, diagnostics and ligand binding studies, folding MP from a denatured state, and MP cell-free synthesis. Various other applications, *e.g.* in proteomics, are currently being developed.
The use of APols to fold full-length MPs that have been either denatured or obtained in an inactive form as inclusion bodies is particularly promising, as it opens a novel route to producing functional MPs whose overexpression is otherwise intractable. Beyond its practical usefulness, it also raises a more fundamental problem. Neither the chemical structure of APols nor their supramolecular organization bear any similarity, beyond their general amphipathy, to that of membrane lipids. Yet, APols constitute a remarkably efficient medium for MPs to fold, even in the complete absence of lipids. Why?
Reading:
Dahmane *et al*. (2009). Amphipol-assisted *in vitro* folding of G protein-coupled receptors. *Biochemistry* **48**, 6516-6521.
Pocanschi *et al*. (2006). Amphipathic polymers: tools to fold integral membrane proteins to their active form. *Biochemistry* **45**, 13954-13961.
Popot, J.-L. (2010). Amphipols, nanodiscs, and fluorinated surfactants: three non-conventional approaches to studying membrane proteins in aqueous solutions. *Annu. Rev. Biochem*. **79**:737-775.
Web site: http://www.ibpc.fr/popot/amphipol/

20-Subg
pH-Triggered Membrane Protein Insertion
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The conversion of a protein structure from a water-soluble to membrane-inserted form is one of the least understood cellular processes. Examples include the cellular action of various bacterial toxins and colicins, tail-anchor proteins and multiple proteins of the Bcl-2 family, bearing pro-apoptotic and anti-apoptotic functions. In our lab we study diphtheria toxin (DT) T-domain, which undergoes conformational change in response to endosomal acidification, inserts into the lipid bilayer and translocates its own N-terminus and the attached catalytic domain of the toxin across the membrane. Our goal is to describe at the molecular level the mechanisms of pH-triggered conformational switching of the DT T-domain, which serves as a model for membrane insertion/translocation transitions of structurally related proteins. Here we present our progress toward this objective, including structural, kinetic and thermodynamic characterization of the insertion pathway of the DT T-domain using both experimental and computational approaches. Supported by NIH GM069783(-04S1).

21-Subg
Transmembrane Helical Protein Folding: Lipid Modulation and Folding Transition States
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General folding principles have emerged from studies on water-soluble proteins, but it is unclear how these ideas will translate to transmembrane proteins, which expose rather than hide their hydrophobic surfaces. We combine kinetic and thermodynamic studies of the reversible unfolding of helical membrane proteins to provide a definitive value for the reaction free energy and a means to probe the transition state.
Efficient systems also need to be developed to stabilise, unfold and re-fold a wider range of alpha helical membrane proteins. We have been